Antibody-functionalized polymer surfaces for local T cell immunosuppression

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Statement of Purpose: The purpose of this work was to synthesize a biomaterial surface that provides T cell-specific immunosuppression. In type I diabetes, a patient's immune system generates autoimmune B and T cells against insulin-producing pancreatic β-cells. Therefore, it is necessary to protect naked β-cell transplants from the recipient's immune system through systemic administration of immunosuppressants. Encapsulating donor β-cells in a hydrogel network has been shown to protect islet transplants from B cell-mediated destruction[1]. Encapsulation alone, however, is inadequate to protect β-cells from small cytotoxic molecules that can easily diffuse into the capsules, such as superoxides and cytokines produced by activated T cells[2]. T cells are regulated *in vivo* via the Fas pathway, whereby cells located in immune-privileged regions express Fas Ligand on cell surfaces. When Fas ligand binds the Fas ligand receptor on a T cell surface, the T cell is triggered to undergo apoptosis. The Fas pathway may be utilized to locally suppress T-cell activation by covalently incorporating DX2 monoclonal antibody, known to induce Fas-mediated T cell apoptosis[3], on a biomaterial surface. Our recent work has shown that modifying a hydrogel surface with DX2 antibody is able to induce T cell apoptosis with moderate efficiency[4]. Because multiple Fas ligand binding events are necessary to activate apoptosis[5], surface mediated polymerization of polymer grafts which cluster DX2 may improve the pro-apoptotic efficacy of the biomaterial surface. The present work focuses on covalently modifying DX2 with acrylate groups such that the acrylated DX2 can be incorporated into poly(ethylene glycol) (PEG) chains through a diethyldithiocarbamyl (DTC)-mediated surface photopolymerization.

Methods: *Acrylation of DX2:* Mouse DX2 and goat (control) IgG were reacted with acrl-$PEG_{3,400}$-NHS. A Fluoraldehyde assay (Pierce, Rockford, IL) was used to assess the extent of acrylation. Acrl-DX2 activity was verified using indirect and direct ELISAs. Indirect ELISA was performed using Goat anti-mouse IgG conjugated to horseradish peroxidase (GAM-HRP) to detect DX2. Soluble Fas receptor was used as the sandwich antigen in the direct ELISA, with goat anti-Fas as the secondary antibody and donkey anti-goat HRP as the detection antibody. *Incorporating antibody into polymer chains:* A base layer was fabricated with 48.5 wt% urethane diacrylate, 48.5 wt% tri(ethylene glycol) diacrylate, 1 wt% tetraethylthiuram disulfide (TED), and 0.5 wt% 2,2-dimethoxy-2-phenylacetophenone (DMPA) as a photoinitiator. 0.25 mg/ml acrl-DX2 or acrl-goat IgG dissolved in 1:1 PEG_{400}acrylate:DMSO was photopolymerized on top of the TED-incorporated substrate for 30-900s under UV light (37 mW/cm^2, 365 nm). Incorporated antibody was quantified using a modified version of the indirect ELISA as described above. *Apoptosis Induction:* Jurkat T cells were exposed to acrl-DX2, DX2, or goat IgG for 24 hrs. The percentage of apoptotic T cells was quantified with an Annexin V apoptosis assay (Molecular Probes, Eugene, OR).

Results: Acrylation of IgG was found to be 14 ± 5 %. A direct ELISA of unpolymerized IgG showed the absorbance of acryl-DX2 to be 61 ± 20 % of unmodified DX2, demonstrating acrl-DX2 maintains recognition of the Fas receptor. Further characterizations of the influences of acrylation on DX2 are underway.

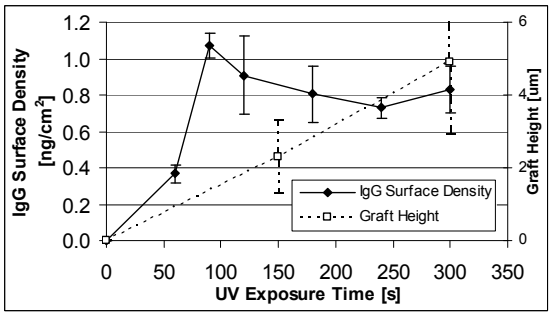

Figure 1. Detectable DX2 & Polymer Height
Acrylated DX2 was photopolymerized from a surface. Available IgG was detected by a modified ELISA; graft height determined by profilometry

Multiple surface polymer conditions were evaluated for maximum antibody incorporation into polymer chains. As shown in Figure 1, UV exposure times ranging from 90-300s produced the highest detectable IgG levels. Further, the detectable antibody increased from 0.92 ± 0.18 ng/cm^2 to 1.65 ± 0.11 ng/cm^2 as the concentration of TED was decreased from 1.0 wt% to 0.25 wt%. Also, detectable IgG increased from 0.55 ± 0.15 ng/cm^2 to 0.78 ± 0.09 ng/cm^2 as the concentration of acrl-IgG monomer was increased from 0.25 mg/ml to 1.0 mg/ml. Preliminary Jurkat cell studies indicated that acrylation of DX2 results in a modest reduction in apoptosis induction ability. Cell work is underway to determine if these surfaces will induce T cell apoptosis and to investigate the role graft antibody density has on apoptosis induction efficiency.

Conclusions: ELISA and cell studies indicated that DX2 maintains the ability to induce T-cell apoptosis following acrylation. Maximum antibody incorporations were found at controlled UV exposure time (90-300s) and TED concentration (0.25wt%). UV exposure times greater than 300s likely resulted in decreased detectable IgG due to high polymer crosslinking and chain transfer. Overall, a novel antibody-functionalized polymer surface was successfully synthesized.

References: [1]Cruise GM, Cell Transplant. 1999;8:293-306, [2]Jang JY, Biomaterials. 2004;25:3663-9 [3]Cifone MG, J Exp Med. 1994;180:1547, [4]Cheung CY, Bioconjugate Chem. 2006;17:1036-1042, [5]Holler N, Mol Cell Bio. 2003;23:1428-1440, [6]The authors would like to thank our funding source: NIH (1RO1DK076084).